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Corn Rootworms: Laboratory Rearing and Manipulation

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Corn Rootworms: Laboratory Rearing and Manipulation

T. F. Branson, P. L. Guss, J. L. Krysan, and G. R. Sutter.[1]

INTRODUCTION

Nearly 10 years ago, Howe and George (*22*)[2] published a procedure for rearing the western, northern, and southern corn rootworms. Since that time rearing techniques have improved, more is known about the laboratory biology of the insects, and obviously from the number of inquiries received, the work of Howe and George has had limited distribution. This bulletin was written to correct these deficiences. The improvement of a rearing procedure is a continuum and some time in the future others will again find necessary the documenting of improvements made in these techniques.

No attempt has been made to review all of the literature pertaining to the rearing of *Diabrotica* spp., but only references are given to those papers that are germane to the rearing procedures described herein.

The northern corn rootworm (NCR), *Diabrotica longicornis* (Say), and western corn rootworm (WCR), *D. virgifera* LeConte, have a similar life cycle. Eggs overwinter in the soil and begin hatching in late spring. Larvae are oligophagous—their host range being limited to certain species of the grass family—but corn is the most important host (*5, 8, 9, 10, 11*). Larvae feed on the roots of corn for several weeks, passing through three instars.

The larval stage is the most destructive causing a reduction in yield through actual damage to the root system and indirectly through lodged plants which are difficult to harvest. Adult emergence begins in midsummer. Adults feed on the aerial portions of the corn plant, specifically the pollen, silks, and leaves, and are known to feed on the flowers and pollen of many other plant species. Adults cause some economic damage by burrowing into the immature ear as they feed on silks, causing poor kernel set. Egg laying begins a few weeks after adult emergence and continues until the beetles are killed by frost (*14*).

The southern corn rootworm (SCR) (or the spotted cucumber beetle), *D. undecimpunctata howardi* Barber, overwinters as a hibernating adult in the southern United States and Mexico. Eggs are laid in the soil in the spring and hatch shortly thereafter. The larvae of this species are polyphagous; they feed on the roots of many plant species including corn, cucurbits, beans, small grains, alfalfa, and grasses.

The immature stages of the SCR are similar in morphology and duration to those of the NCR and WCR. The adult can cause economic damage to many garden and field crops including corn, cucurbits, and legumes. The SCR has three generations in the south (*1*) and at least a partial 2d generation in the north (*27*).

ADULTS

Collection

Adults of all species can be collected from fields of corn and cucurbits by sweeping weedy undergrowth, by collecting from flowers using various vacuum collectors, and by knocking insect-laden tassels and ears against a funnel collecting device. One of the most efficient devices for collecting adults is a small battery-powered vacuum cleaner which has been modified by adding a screw-lid jar and a short polyethylene tube for collecting (fig. 1). With this type of equipment, this laboratory collects 50,000 to 100,000 beetles annually by aspirating them from the ears and tassels of corn plants.

Beetles are intolerant to high temperatures and will die in hours without moisture. Therefore, adults being held in the field or being transported to the

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[2] Italic numbers in parentheses refer to Literature Cited, p. 17.

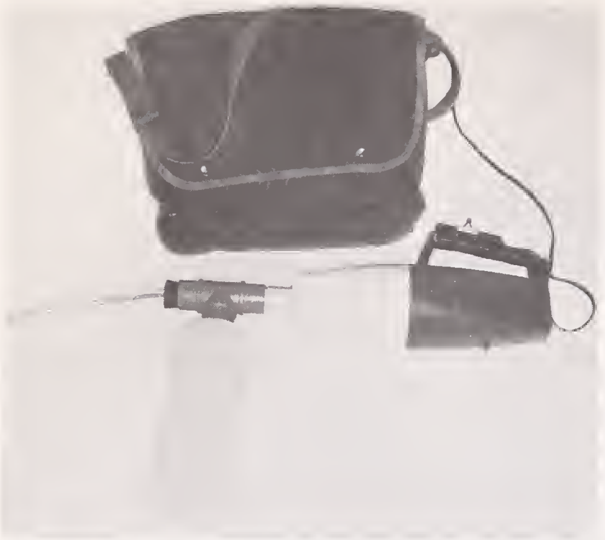

FIGURE 1.—Portable vacuum cleaner modified for collecting adult corn rootworms.

laboratory must be kept cool, well fed, and watered. This can generally be accomplished by keeping the beetles in the shade and feeding them immature corn ears from which the husks have been opened or removed.

Southern corn rootworm and WCR adults can be retained in 16-mesh cages. However, because the NCR is smaller, cages with a mesh size of at least 18 must be used. Beetles can be held for short durations at a population density of 2,000 beetles per 1 ft^3 (0.028 m^3) of cage; however, the density should be about 750 to 1,000 beetles per 1 ft^3 (0.028 m^3) of cage if they are going to be held for more than a day.

Timing of collections in the field is critical for optimum egg production in the WCR. If collections are made too early, egg production is reduced by having a predominance of males, since males emerge several days earlier than females. If collections are made too late, peak egg production may be missed, poor food quality in the field may result in unfecund beetles, and the population may have dispersed and therefore be more difficult to collect. Therefore, for optimum egg production, collections of WCR should be made within 1 to 2 weeks after females first begin to appear in the field (*7*).

Maintenance

Adults are maintained at 30 percent RH, 24° to 27° C, and a photophase of 12 hr with subdued and indirect lighting. Beetles are held in cages of 16 mesh (SCR and WCR) or at least 18 mesh (NCR) at a population density of about 750 to 1,000 per 1 ft^3 (0.028 m^3) of cage. Before use and between populations, the cages should be scrubbed clean of dead beetles and frass and sterilized by soaking in a 10 percent bleach solution or by autoclaving.

Dead beetles should be removed daily because they can be a source of inoculum for insect diseases; and the walls, floor, and ceiling of the rearing facility should be scrubbed weekly with an aqueous solution of detergent and such an antiseptic as bleach. The use of a central vacuum system for cleaning cages and the daily removal of dead beetles will help to control insect diseases.

Beetles can be immobilized by chilling or by anesthetizing them with CO_2 (*12*). Carbon dioxide causes the beetles to regurgitate, but inactivates them for a longer time than does chilling. A simple chamber for anesthetizing adults with CO_2 can be made by gluing one plastic petri dish on top of another, drilling holes in the bottom of the top dish, and attaching a glass tube to the side of the bottom dish (fig. 2).

Adults can be fed immature ears (blister stage) of corn from which the husks and silks have been removed. Immature ears can be picked in the fall and stored in the husk for several months under refrigeration. Ears in the soft dough stage can also be fed if they are first slashed several times, longitudinally, to the cob. Allow about 2 ears per 1,000 beetles, renewed twice per week. Oviposition is increased greatly when the ears are laid over the oviposition site.

Adult food can also consist of fresh seedling leaves of corn (SCR, WCR, and NCR) or the fresh leaves of broad beans, *Vicia fabae* L. (SCR). Fresh leaves, cut from plants, are bundled together, with a rubber band around the stems to form a bunch about 2 inches (5 cm) in diameter at the stem end (fig. 3). Again, egg laying is improved by laying this bundle over the oviposition site.

One bundle is fed to each 1,000 beetles per day. This diet is probably suboptimal nutritionally and, although a colony can be maintained exclusively on it, egg production will be improved by feeding, in addition, a mixture of pollen substitute and honey (*21*). Pollen substitute, a mixture of soybean meal and brewer's yeast, is available from beekeeper's supply houses.

Artificial Diet

Agar-based artificial diets for WCR adults have been describd by Singh and Howe (*30*) and Cuthbert and others (*15*). Studies at this laboratory with agar-based diets for SCR adults showed that the diet used for SCR larvae (*33*) was acceptable to the adults, but fecundity and longevity were reduced.

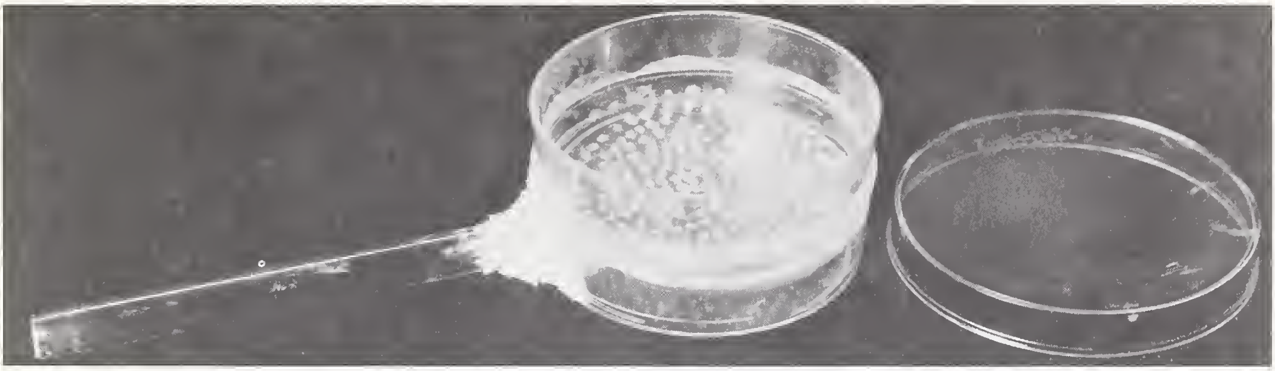

FIGURE 2.—Chamber for anesthetizing adult corn rootworms with carbon dioxide.

Although agar diets for rootworm adults represent an improvement in ease of maintenance over diets consisting of fresh foliage, they are not without some inherent problems. Agar diets must be changed daily because of desiccation, and the limited storage life of these diets does not permit large batches to be made up long before their intended use. Another disadvantage of agar-based diets is that they must be protected from contamination by the addition of antibiotics or other microbial inhibitors. Some of these compounds could have an undesirable effect on eggs and larvae from adults maintained on agar diets.

Guss and Krysan (*19*) developed a dry formulated diet for adult SCR which, with minor modification, is also suitable for WCR adults. The diet consists of a mixture of nutrients, vitamins, and minerals combined with a high protein bulk ingredient (beekeeper's pollen substitute) in about equal proportions (table 1). All the ingredients are mixed together dry, and for the SCR the diet is fed as is.

Because no water is used in the diet, the beetles must be provided with a moisture source. For this, one can use glass vials 50 by 180 mm filled with water and fitted with a rubber stopper through which is threaded a cotton wick (fig. 4). If these wicks are not changed every other day, they become contaminated by bacteria. This problem can be partially alleviated by substituting flat beer for the water. Although the beer is not so subject to bacterial contamination, the wicks still must be changed regularly (every 3 or 4 days) to avoid fungal growth.

As formulated by Guss and Krysan (*19*), the diet is entirely suitable for SCR adults; however, for the less robust WCR adults, the physical properties of the diet

TABLE 1.—*Ingredients for dry formulated diet for rootworm adults*

Additives	Weight
	Grams
Beekeeper's pollen substitute	290
Wheat germ	54
Casein	63
Sucrose	48
Alphacel	9
Salt mix W	18
Vitamin mix[1]	18
Cholesterol	.125

[1] Vanderzant modification vitamin mixture for insect diet (Nutritional Biochemical, Corp.).

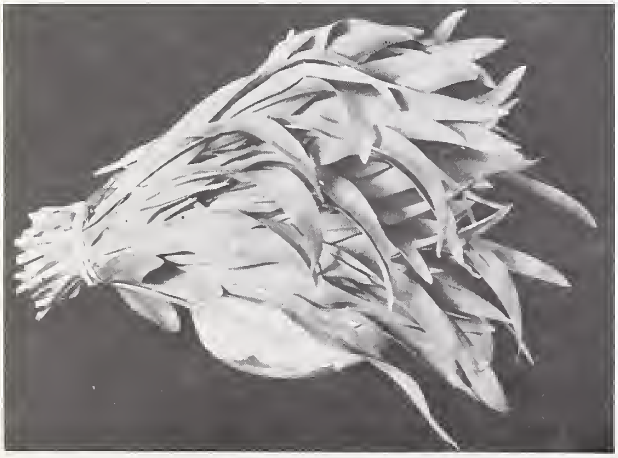

FIGURE 3.—A bundle of adult food covering an oviposition site.

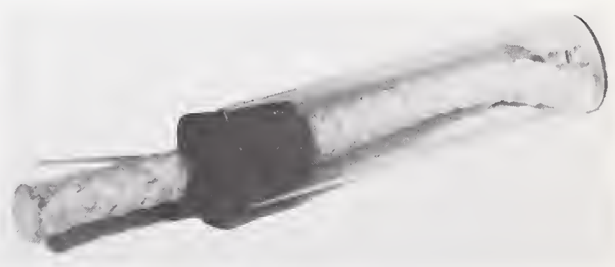

FIGURE 4.—Moisture source for adult corn rootworms used with the artificial dry diet.

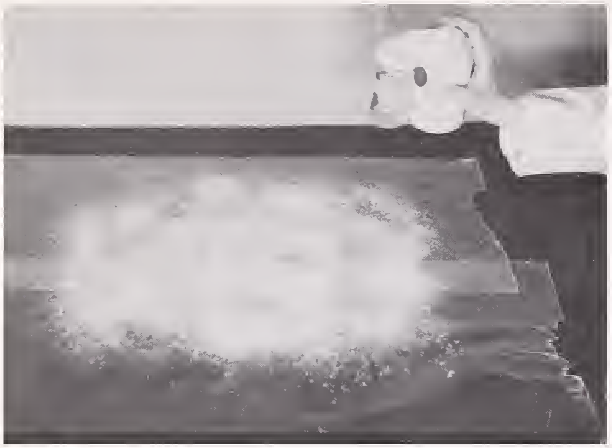

FIGURE 5.—The process of granulating artificial dry diet for adult western corn rootworms.

must be modified as follows: The diet is ball-milled overnight to form a fine powder. The ball-milled diet is then spread thin on waxed paper sheets and moistened lightly by a fine water mist (fig. 5). The diet is allowed to air dry after which it is ready for use with WCR adults.

The ball-milling is necessary to make the more bulky ingredients (notably the wheat germ) accessible to the WCR adults, and the spray procedure is essential to give the diet enough structural stability to avoid entrapment or coating of the beetles with fine dust. At this laboratory where both the SCR and WCR are reared, the modified diet is used routinely for both species. When only the SCR is involved, modifying the diet is not necessary.

The diet can also be fed in pill form by first subjecting the ball-milled dry diet to a pressure of 2000 lbs/in^2 in a pill die. This is a particularly useful method for feeding adults which will be shipped long distances.

The dry diet for rootworm adults has a number of advantages over both fresh foliage and agar-based diets. The diet can be made up in large quantities and stored indefinitely because the possibility of deterioration during storage is virtually nonexistent. The dry formulation also resists reproduction by microorganisms; thus, antibiotics or other preservatives are unnecessary. Of distinct advantage from a research point of view is that eggs originating from rootworm adults fed the dry diet are exceptionally clean.

Eggs from adults fed a fresh foliage diet are difficult to clean of debris originating from the plant material and adhering to the chorion. Use of the dry diet greatly simplifies maintenance of rootworm colonies. Because deterioration is no problem, the diet does not have to be changed until the diet containers become so encrusted with frass that they must be cleaned—usually once or twice per week. General sanitation is greatly improved over natural diets.

Valuable greenhouse space, needed for the production of natural food, is freed for other purposes. A cost comparison of the exclusive use of dry diet versus natural diets for maintenance of rootworm adults at this laboratory showed that a reduction of over 98 percent of the cost of maintaining adults could be realized with the dry diet.

Sexing Adults

Adults can be sexed by comparing the size of antennal segments or by morphological differences in the terminal segments of the abdomen. Individual antennal segments are larger in the male than in the female (fig. 6). The terminal segment of the female abdomen is acute and that of the male is rounded in general conformation. The difference is caused by an additional rounded sclerite terminating the abdomen of the male; the sclerite does not exist in the female (*26*) (fig. 7).

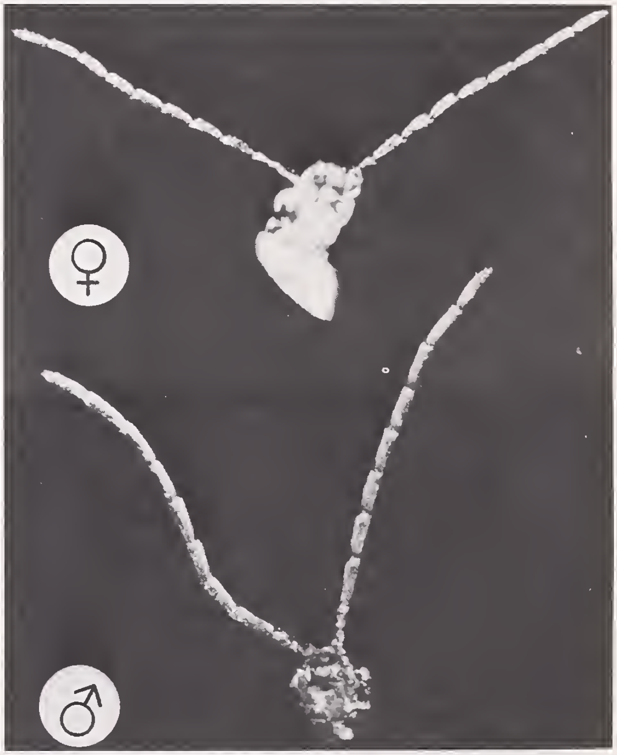

FIGURE 6.—Antennae of female and male corn rootworms. Individual antennal segments are larger in the male than in the female.

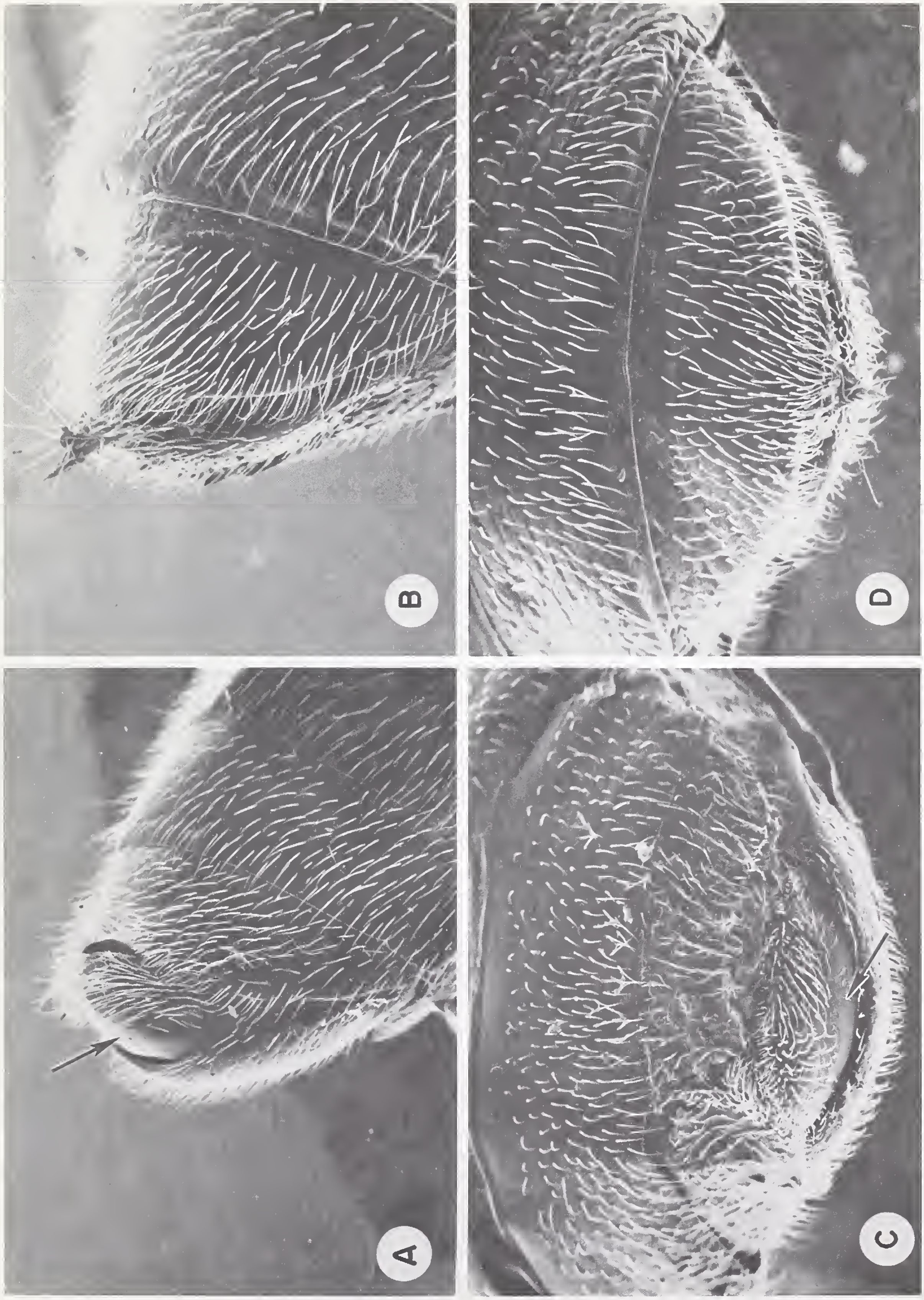

FIGURE 7.—Terminal segments of the abdomens of female and male corn rootworms: A, male—side view; B, female—side view; C, male—end view; D, female—end view. Arrows indicate the rounded sclerite terminating the abdomen of the male. (Scanning electron micrographs courtesy of Dr. Donald Hostetter, ARS, USDA, Columbia, Mo.)

Mating and Oviposition

A particular male/female ratio need not be maintained within a cage because SCR males copulate repeatedly (*1*) and WCR males seem capable of repeated copulation. Female WCR need to be mated only once (*7*), but there seems to be some difference of opinion concerning the mating habits of the female SCR. Arant (*1*) states that the female SCR mates only once; yet Skelton and Hunter (*31*) present data which indicate that repeated mating may be necessary to insure a high level of egg viability.

Copulation has been observed between teneral WCR adults (*13, 2*) and periodic mounting, at least, has been observed throughout the entire lives of adult WCR, but it is not known at this time when viable sperm is actually passed to the female. Therefore, we must assume (until this question is answered) that WCR females are mated when they first emerge from the soil. The only certain way to obtain virgin WCR adults is to cloister individuals when they are pupae. Copulation between SCR adults reportedly does not occur until 5 to 18 days (a mean of 9 days) after emergence (*1*).

Oviposition media can be either gauze, crumpled to form crevices and dampened (fig. 8) or soil sifted through an 80-mesh screen and dampened (fig. 9). The oviposition medium must be kept damp, but not have standing water. Beetles become acclimated to one medium, so if the medium is changed from one form to another, there will be 1 to 2 days before normal egg production is resumed. There should be one oviposition site (100 by 15 mm plastic petri dish) per 1,000 beetles. Oviposition sites of WCR and NCR are changed twice per week, and those of SCR are changed three times per week.

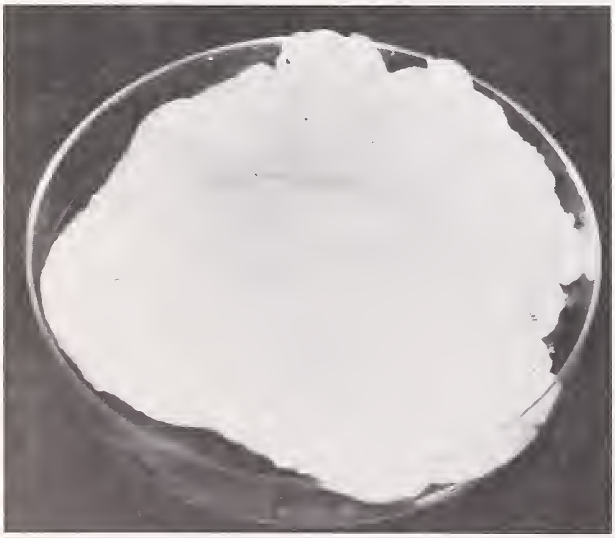

FIGURE 8.—Crumpled and dampened gauze as an oviposition medium.

FIGURE 9.—Oviposition medium of soil that has been sifted through an 80-mesh screen and dampened.

Egg production is reduced by keeping beetles in strong direct light and by keeping the oviposition sites uncovered. Beetles prefer to oviposit beneath an object so oviposition sites are covered with food (fig. 3) when plant material is used; or by stainless steel flutes (fig. 10) when dry diet is used. Oviposition sites and eggs covered with stainless steel flutes are kept considerably cleaner than those covered with fresh food. To mitigate against disease problems, dead beetles should be removed daily from the oviposition site. Ball (*2*) indicates that maximum oviposition by WCR occurs during the relatively early part of the photophase.

The average WCR female should survive for about

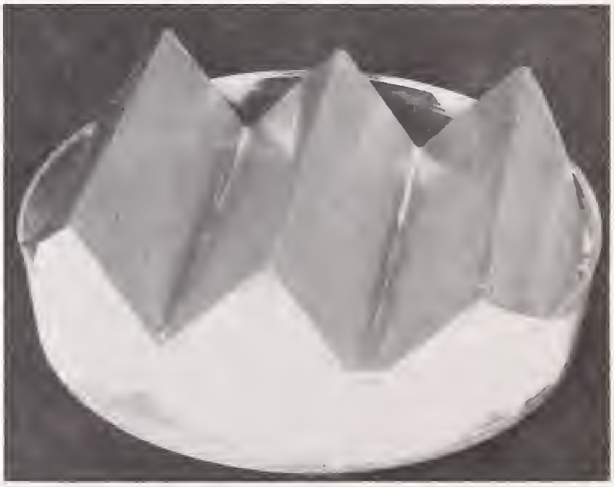

FIGURE 10.—Flute of stainless steel covering an oviposition site.

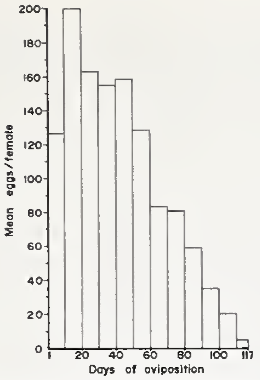

FIGURE 11.—Distribution of eggs laid by western corn rootworms throughout the oviposition period.

3 months (females live longer than males) and lay about 1,000 eggs. Ninety-five percent of all the eggs a given WCR population is capable of laying will be laid within 90 days after oviposition begins (*7*). Therefore, any surviving members of a WCR population should be destroyed 90 days after initiation of oviposition because to continue caring for them would be inefficient in terms of cost per egg return and would increase the probability of disease problems. Female WCR have a preoviposition period of 2 to 4 weeks, oviposition begins at a high level, peaks about 10 to 15 days later, and slowly declines (fig. 11). Healthy WCR females can be expected to lay a mean of 12 eggs per female-day during the reproductive period (*7*).

Females of SCR have a 10- to 20-day preoviposition period. The pattern of oviposition is not known for this insect, but Howe and George (*22*) indicated that 28 females laid over 6,000 eggs the first 28 days of oviposition. This amounts to about 8 eggs per female-day during the first month of oviposition. Skelton and Hunter (*31*) found the average SCR female laid more than 700 eggs in the first 65 days after mating, and Guss and Krysan (*19*) found SCR beetles laid about 20 eggs per female-day over 15 days. Arant (*1*) indicates that adult SCR live about 70 days and that females live longer than males. Although the longevity of female SCR has never been measured at this laboratory, we believe that they are capable of living considerably longer than 70 days.

Shipping

Adults can be shipped, with proper permits, by providing them fresh leaves as food when they will be in route for only a day or two. When they will be in route for longer periods, they should be provided dry diet, in pill form, and a non-leaking water source. A non-leaking water source can be made by rolling a wick from a piece of absorbent paper and inserting it through a tight-fitting hole in the rubber bulb of a medicine dropper which has the glass pipet removed (fig. 12).

Aircraft on international flights are fumigated with pyrethrin insecticide which will destroy any adults being shipped. This treatment can be overcome by sealing the beetle container in a sheet of polyethylene. Pyrethrins do not penetrate the plastic barrier, yet sufficient passage of oxygen will be allowed to insure survival.

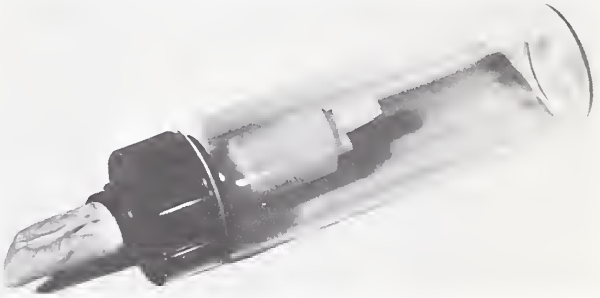

FIGURE 12.—Non-leaking water source for use when shipping adults for long distances.

EGGS

Identification

Sculpturing of the chorions of the three species of corn rootworms can be seen in fig. 13. Note that eggs of the WCR can be differentiated easily from the eggs of either the NCR or the SCR. But discriminating between the eggs of the NCR and the SCR is difficult (*29*).

Handling

The egg stages of the NCR and WCR vary greatly from that of the SCR. Few difficulties are encountered in handling the eggs of SCR. They are separated from the cheesecloth oviposition medium by washing over a 60-mesh screen and placed on dampened filter paper for hatching. If sifted soil is the oviposition medium, eggs can either be washed from the soil through a 60-mesh screen and placed on filter paper for hatching or hatched *in situ*.

Eggs are placed in a closed container and kept damp until hatch. At 25° C hatching will begin in 7 days and will be completed in about 10 days. One can expect a hatch of SCR eggs approaching 100 percent. Eggs of the SCR can be stored up to 30 days at 5° with a slight loss of viability (*22*).

Eggs of the NCR and WCR are quite a different

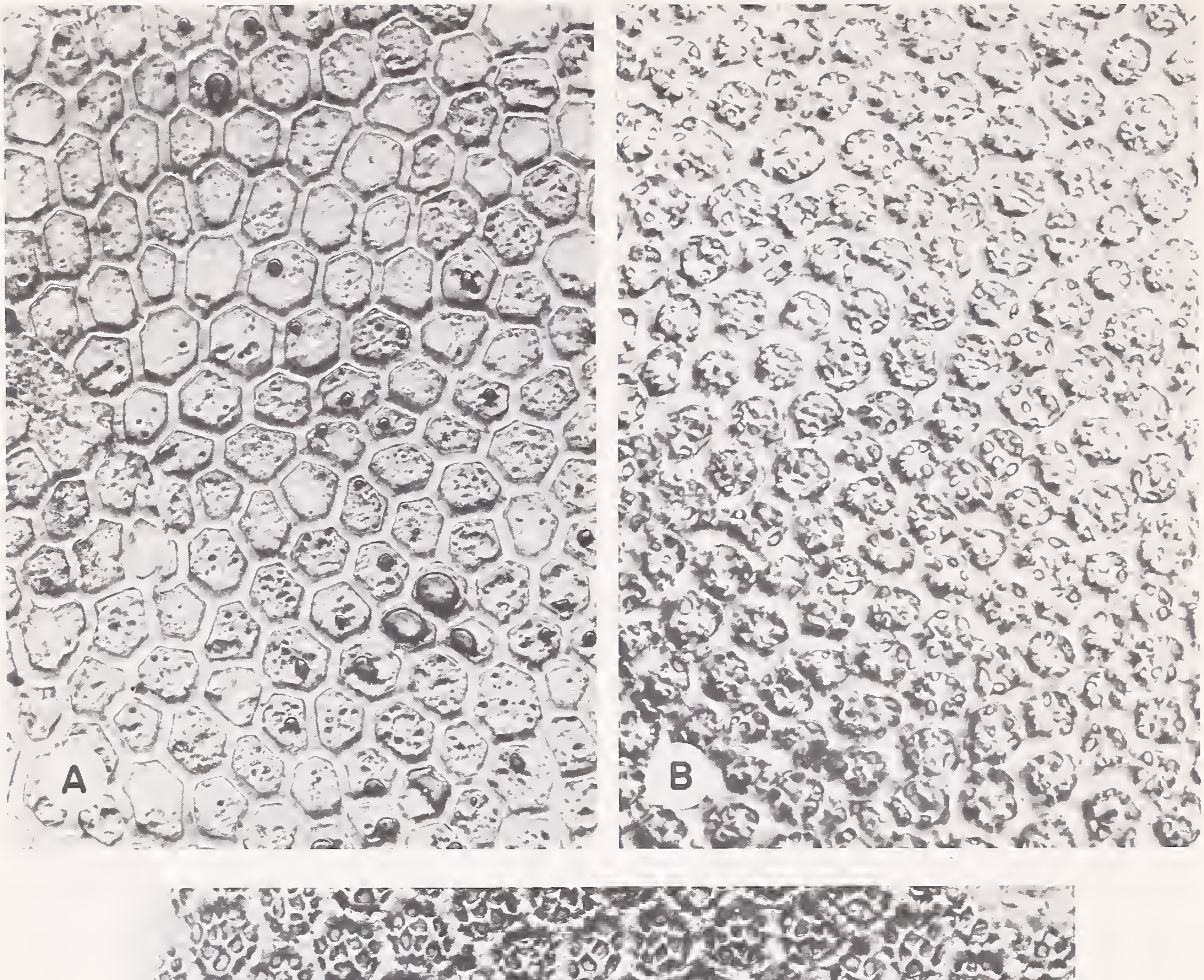

FIGURE 13.—Sculpturing of the chorions of corn rootworms: A, western corn rootworm; B, northern corn rootworm; C, southern corn rootworm.

matter: eggs of these species have a diapause which makes handling something of a problem. Eggs laid in sifted soil are normally kept damp and left *in situ* for hatching; eggs laid in cheesecloth should be washed over a 60-mesh screen and placed into soil sifted through an 80-mesh screen or washed onto filter paper for hatching. Because the eggs must be left for a long time before hatching occurs, mold growth can become a serious problem when eggs are stored on filter paper. Mold problems can be lessened considerably if the eggs are kept clean and free from debris and if the paper has been boiled three times in three changes of water (R. D. Gustin, personal communication).[3]

Mold growth does not appear to be a problem when eggs are laid in sifted soil and stored *in situ*. Eggs must be kept damp (not wet) throughout the storage period. This can be accomplished by moistening the eggs well just before they are placed in storage, inserting a piece of plastic between the lid and bottom of the storage container (usually a 100 by 15-mm plastic petri dish), taping the lid down, and sealing 10 to 15 egg dishes in a plastic bag.

Eggs are easily counted by washing them into a Büchner funnel containing filter paper marked with a grid. Handling eggs naturally reduces their viability; however, eggs do become tougher and more tolerant to handling with age.

Eggs can be removed from field-collected soil by washing the soil and floating the eggs off in a saturated solution of epsom salts or sucrose. Various mechanical devices have been developed to perform these tasks automatically. One developed by the Illinois State Natural History Survey has been quite effective. Eggs that are recovered from soil in this manner retain their viability.

Hatching

For hatching, WCR eggs are routinely treated to 2 weeks of pre-chill at 22° to 25° C. Eggs are then chilled at 5° for 120 days, then incubated at 20° to 25° until hatch. Initial hatch occurs in 14 to 24 days after chilling and is usually completed 40 days later. Peak hatch of WCR eggs incubated at 20° to 22° occurs in the first week of hatching with 98 percent of the egg hatch occurring in the first 3 weeks (fig. 14). There is, therefore, little reason to retain WCR eggs for more than about 3 weeks after the initiation of hatch.

Chilling does *NOT* break diapause in eggs of WCR.

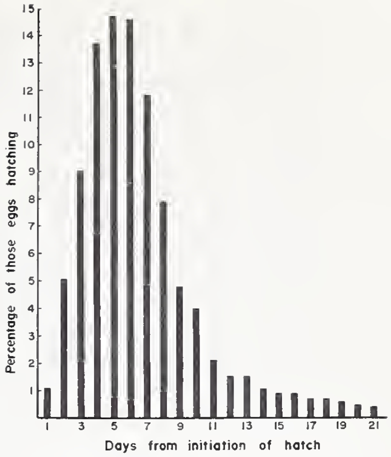

FIGURE 14.—Hatching pattern of eggs of the western corn rootworm after chilling at 5° C for 120 days.

The passive consequence of chilling is a synchronization of hatch. George and Ortman (*18*) reported that eggs of WCR hatched without chilling, but that the hatching period was considerably longer than when eggs were chilled first and then incubated. Krysan's (*25*) data indicates eggs of WCR and NCR have an embryonic rudiment immersed in the yolk at diapause. Prediapause eggs develop to the diapause stage either before or during chilling and they remain in the diapause stage during chilling (apparently, they are unable to develop beyond this stage at 5° C). Postchill eggs then develop rapidly and in relative synchrony until hatch.

Initial hatch of unchilled eggs of WCR occurs in about 5 weeks, peak hatch occurs at about 16 weeks, and hatch continues at a slow rate for more than 30 weeks. Selection of a nondiapause strain of *D. virgifera* can be accomplished by mass selection.

Determining the percentage of eggs hatching *in situ* does not seem possible; however, one can expect about 50 percent hatch with eggs of WCR washed either into soil or onto filter paper. The percentage hatch does not appear affected by the presence or absence of a chill period (of 5° C); nor does hatchability appear to be affected by chill periods of less than 120 days. However, eggs of WCR show a gradual loss of viability when stored at 5° for more than 120 days. About an 80 percent reduction in hatch is expected when WCR eggs are stored for 1 year. In other words, eggs that have a hatchability of 50 percent after 120 days at 5° will have a hatchability of only about 10 percent after 1 year at that temperature.

Screening for Hatchability

For determining hatchability of egg populations following various treatments and for screening potential ovicides, eggs are placed on a sterile 1⅞-inch blotter paper disk (25 eggs per disk) and put in a 20 by 60-mm plastic petri dish containing 1 ml of water or an aqueous solution or suspension of the chemical to be screened. Dishes are then sealed with Parafilm and incubated at 25° C.

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[3] R. D. Gustin, unpublished communication. Northern Grain Insects Research Laboratory, ARS USDA, Rural Route 3, Brookings, S. Dak. 57006

Hatch can be determined for eggs of WCR by counting the empty chorions at the end of the hatching interval (usually about 25 days). Newly hatched larvae of the SCR will, especially if crowded, eat unhatched eggs and therefore they are routinely counted and removed from the dishes daily. The dishes are then resealed. Generally, there is no need to add water to the dishes.

At times, microbial contamination of various sorts becomes a problem. As stated earlier, boiling the pads three times in water greatly reduces this problem. Several common fungistatic and fungicidal materials have been examined by incorporating them into the system. Of these, methyl paraben and 8-quinolinol sulfate (the fungicide used for treating the corn used as larval food—see following section on larvae and pupae) greatly reduced hatch of WCR eggs. Sodium sorbate and the fungicide 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole permitted normal hatch of WCR eggs. Whether these compounds would prove to be useful in any particular instance would depend on the sensitivity of the microbial contaminants involved.

LARVAE AND PUPAE

Transferring Larvae From Egg Dishes to Rearing Medium

Eggs of the SCR can be added directly to the larval rearing medium when they begin to hatch. This is normally accomplished by washing the larvae and unhatched eggs from the egg dishes onto the rearing medium with a plastic wash bottle (fig. 15).

However, hatching of a population of WCR and NCR eggs is extended over such a long duration that unhatched eggs cannot be added to the rearing medium. Newly hatched larvae are picked from the egg dishes with a small, moist brush, placed on damp filter paper in conveniently sized batches, and washed onto the rearing medium. A kernel or two of germinated corn treated with fungicide (at this laboratory a fungicide with 8-quinolinol sulfate as the active ingredient is used) and placed in each egg dish will attract the larvae as they hatch and much time time spent picking individual larvae can be saved by simply transferring the larva-infested kernel to the rearing medium.

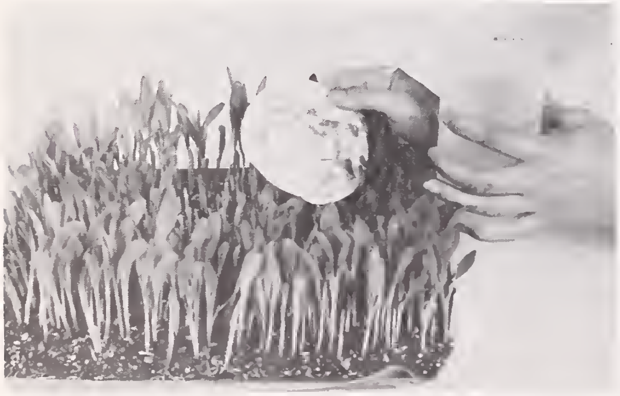
FIGURE 15.—Washing larvae and unhatched eggs of the southern corn rootworm from egg dishes onto the rearing medium.

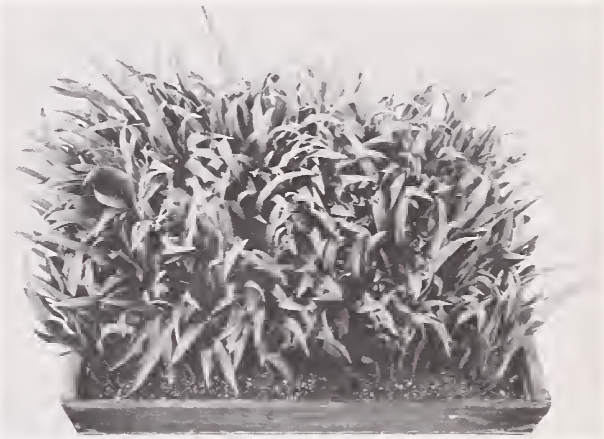
FIGURE 16.—A mat of seedling corn used as larval rearing medium.

Also, newly hatched larvae tend to show either positive geotaxis or negative phototaxis, or both, so, if the egg dishes are turned upside down, the larvae will then congregate on the lid of the egg-dish where they can easily be washed onto the rearing medium. Larvae must be removed from the egg-dish daily because their chance of survival improves when they have immediate access to food.

Rearing Conditions

The immediate larval rearing medium is a mat of seedling corn roots (fig. 16). The mat frame is 9 by 15 by 1¾ inches deep (23 by 38 by 4.5 cm) with a bottom of ¼-inch (6.4-mm) hardware cloth. To prepare the mat, the bottom of the frame is lined with plastic sheeting through which two ½-inch (13-mm) holes have been punched for drainage. The plastic sheet is covered with a layer of a 1:1 mixture of potting soil

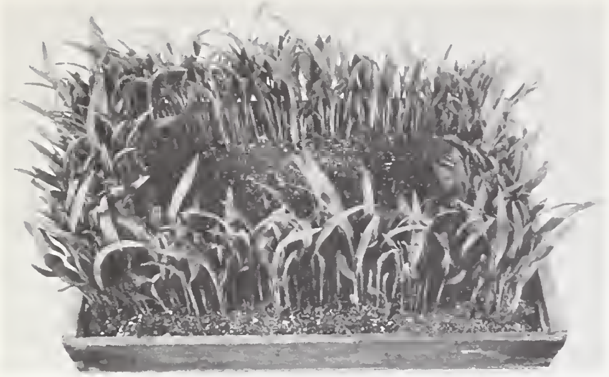

FIGURE 17.—Greenhouse flat with corn planted around the margins; used as larval rearing medium and pupation site.

and vermiculite, a layer of untreated corn seed is added, and the mat frame filled with the soil vermiculite mixture.

Each mat is infested with 200 to 250 larvae when seedling corn is very young, about 1 inch (2.5 mm) out of the soil. After 14 days, the entire mat is transferred to a greenhouse flat 15 by 23 by 4 inches (38 by 58 by 10 cm) containing corn plants [2-4 inches (5-10 cm) tall] around the margins of the flat (fig. 17). A depression is left in the center of the flat, and the entire mat is lifted from the plastic sheet and placed into this depression. Survival of the larvae is improved by the periodic addition of a handful of germinated corn (treated with a fungicide) under each mat. The flats are caged for adult emergence about a week after transfer of the mat (fig. 18).

Under the conditions at this laboratory, WCR and SCR adults begin to emerge about 35 days after infestation as larvae, but this is highly variable and greatly dependent upon temperature. The percentage return from larvae to adults varies considerably among flats, but over several years the return of WCR has averaged about 50 percent.

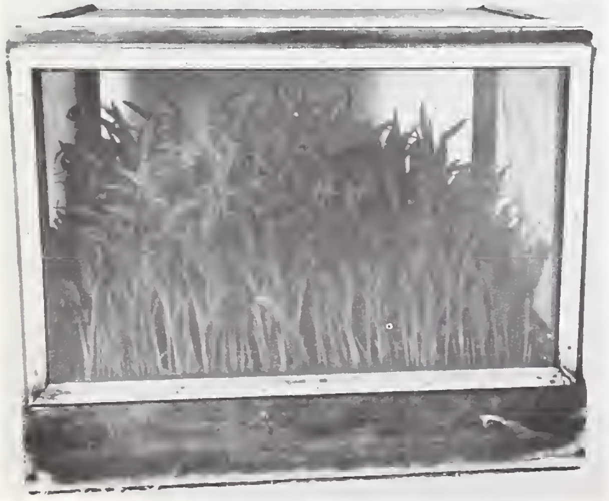

FIGURE 18.—Flat caged for adult emergence.

Proper watering of the flats is critical for optimum survival of the larvae. Most people have a tendency to overwater at this stage, causing the corn to rot and the larvae to die from disease or starvation. The only way to be certain whether a flat requires water is to probe the soil with a finger. If any moisture can be felt, then the flat should not be watered.

Larvae are reared in the greenhouse at 24° to 27° C. Some form of cooling and shading is necessary in the summer and supplemental lighting is necessary at northern latitudes during the winter. Cutworms will frequently contaminate the larval rearing media. Moths are able to penetrate the tightest greenhouse and lay their eggs on the seedling corn in the flats. Cutworm larvae hatching from these eggs destroy the plants rapidly. Commercial bait containing *Bacillus thuringiensis* scattered in the flats will eliminate cutworm larvae without harm to rootworm larvae or adults (*32*).

When larvae are required for experimental purposes, they can be easily extracted at any stage from mats or flats by Berlese funneling. A mat or flat is emptied on a ¼-inch (6.4-mm) hardware cloth screen suspended over water. A light bulb over the soil will, in a few hours, drive the larvae down, forcing them to fall into the water where they can periodically be skimmed from the surface.

If larvae must be observed while they are feeding or being reared, they can be reared on corn seedlings growing on agar in petri dishes (*4*) or on corn seedlings growing in plastic growth pouches[4] (*6*). However, either method will require that larvae be transferred to new material about every 10 days. These are highly artificial systems and with them mold growth is a constant problem, but these techniques have some experimental usefulness.

Artificial Diet

Artificial agar-based diets have been developed and used successfully for rearing larvae of the SCR from 1st instar to adult (*33, 28*); however, attempts at formulating similar preparations for larvae of the WCR have not been successful possibly because the proper combination of feeding stimulants (or arrestants) is not known. Branson and Ortman (*8, 10*) demonstrated that larvae of the WCR are quite restricted in their host range whereas larvae of the SCR are apparently able to mature on a number of

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[4] diSPo growth pouch. Scientific Products, Div. of American Hospital Supply Corp., 1210 Leon Place, Evanston, IL 60201

TABLE 2.—*Artificial diet for southern corn rootworm larvae*

Component		Amount
A: Distilled water	milliliters	3360
Agar (Difco)	grams	58
B: Wheat germ	do	110
Casein	do	129
d-Sucrose	do	129
Alphacel	do	55
Salt mix W	do	37
Methylparaben	do	4
Sorbic acid	do	2.5
Cholesterol	do	.25
Raw linseed oil	milliliters	1.0
C: Streptomycin	gram	.5
Achromycin	do	.5
Vitamin mixture[1]	milliliters	60.0
10% KOH	do	40.0
Formalin	do	4.0
Total	grams	4000

[1] Nutritional Biochemical Corp.

substrates including roots of cucurbits, peanuts, and corn (*1*).

The recipe for the artificial diet used for SCR larvae at this laboratory is contained in table 2. The agar is dissolved in water by heating in an autoclave for 20 minutes. The agar is then placed in a blender at low speed while the ingredients in group B are added. After mixing at high speed for 1 min, the diet is cooled to 40° to 45° C and the components in group C are added. The complete diet is then mixed 2 min and dispensed into 1-oz plastic cups (about 10 ml/cup). The recipe in table 2 will yield about 300 cups of diet.

After solidification, 5 to 10 1st-instar SCR larvae are added to each cup of diet, and the cups are capped with unlined paper lids. Incubation in total darkness for 13 days at 26.5±½° C and 75±5 percent RH was sufficient for complete larval development.

Southern corn rootworm larvae cannot be allowed to pupate in this diet because they tend to burrow into the diet to form pupation cells which eventually cause entrapment of the adults upon emergence. Thus, when the SCR larvae reach 3d stage, the capsules (with lids removed) are placed on clay bulb pots containing a 2:1 autoclaved mixture of damp vermiculite and sand (fig. 19). A 32-cm diameter clay pot filled with 2½ liters of sand-vermiculite mixture can accommodate about 1,000 larvae.

After 24 hrs, most of the larvae have migrated from the diet into the sand-vermiculite mixture, and the diet cups are discarded. Each pot is then covered with a ventilated plexiglass lid and incubated at 26.5° C and 75 percent RH. On alternate days until emergence is completed, 400 ml of water are added to the saucer of each pot.

Rearing rootworm larvae on an artificial medium has several advantages. The principal advantage from a research point of view is the easy access to all the immature stages afforded by this method. This fact will be apparent to anyone who has had occasion to isolate rootworm larvae or pupae from cultures reared in soil. Other advantages include: (1) the ability to feed accurate amounts of experimental reagents to larvae and to observe their subsequent reactions; (2) a decrease in space over that required by the "root-mat" method; and (3) a significant decrease in time and labor necessary to maintain a rootworm culture.

This method is not without some disadvantages. For example, Sutter and others (*33*) experienced erratic and occasionally severe contamination problems with the diet. The most likely source of contamination was the larvae themselves, but attempts to surface sterilize eggs of the SCR with Hyamine 10-X, Hyamine 1622, Clorox, and Zephiran chloride in concentrations sufficient to reduce contamination resulted in greatly reduced egg viability and larval vigor, the latter being especially important for establishment on the diet.

The only material showing promise as an egg surface sterilant was peracetic acid used according to Doll and others (*16*). Southern corn rootworm eggs soaked for 5 min in a 1 percent aqueous solution of peracetic acid containing 0.075 percent sodium alkyl aryl-sulfonate had normal viability and did not cause contamination when placed directly on the artificial

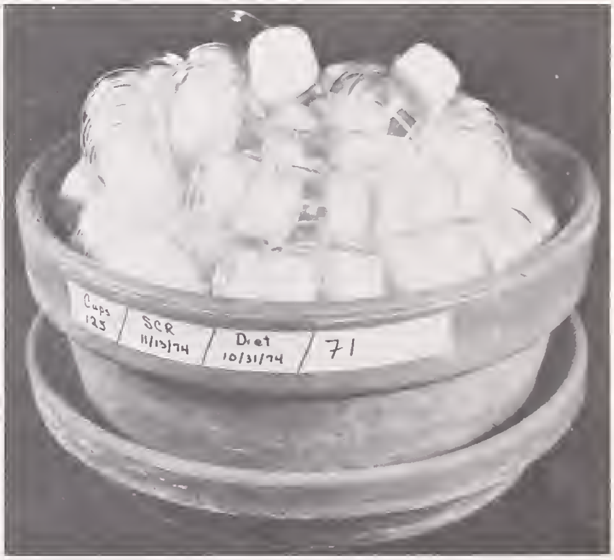

FIGURE 19.—Pupation site for southern corn rootworm when they are reared on artificial diet.

diet. The procedure has not been incorporated into the protocol for rearing southern corn rootworms on artificial diet because that diet is used intermittently, a pattern of use that has not led to serious contamination.

Another possible disadvantage of this method is that rootworm larvae reared on an artificial medium for several generations may become so adapted that their usefulness in such programs as host plant resistance might be seriously impaired.

Laboratory Biology Of Immature Rootworms

The duration of each stadium is extremely variable and highly dependent upon temperature and the availability of food. However, on the whole, one can expect that about 16 to 17 percent of the immature period is spent as a 1st instar, 16 to 21 percent as a 2d instar, 37 to 45 percent as a 3d instar, and 22 to 26 percent as a pupa. If the insects require 35 days to mature from hatchlings to adults, then the 1st larval stadium would last about 6 days, 2d 6 or 7 days, 3d 13 to 16 days, and the pupal stage would last 8 or 9 days (*17, 1, 23, 34*).

Three to six days before pupation, the mature 3d instar ceases to feed, locates a suitable place to pupate, and assumes a c-shaped, quiescent prepupa (fig. 20). The pupal stage is revealed when prepupal skin is cast off (fig. 21). Female pupae have prominent papillae on the venter of their abdomen (fig. 22) (*17*), so the pupal stage is more accurate than the adult stage for sexing individuals.

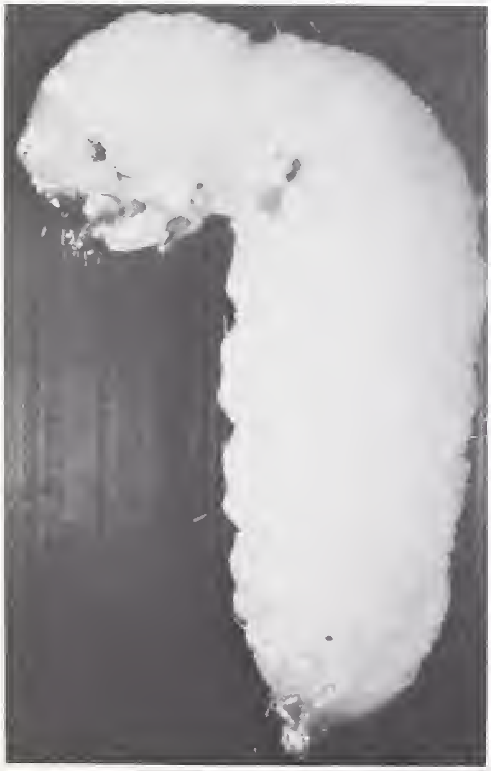

FIGURE 20.—Prepupa of corn rootworm.

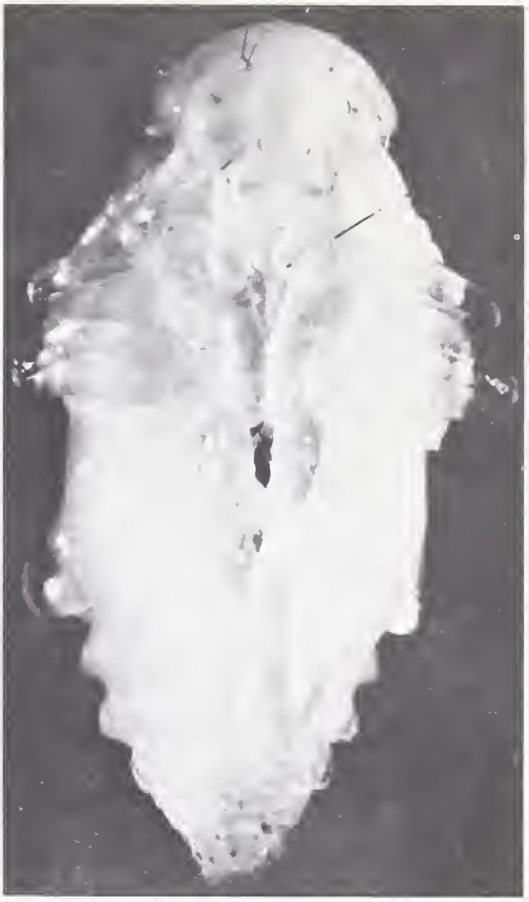

Figure 21.—Pupa of corn rootworm.

George and Hintz (*17*) reported the head capsule measurements of larvae of WCR:

Instar	Extremes of head capsule width
	Microns
1st	200-225
2d	300-350
3d	450-500

For SCR larvae, Arant (*1*) gave the following head capsule measurements:

Instar	Extremes of head capsule width
	Microns
1st	250-300
2d	390-415
3d	580-640

The SCR larvae at this laboratory appear somewhat larger than this; however, Arant's measurements can be utilized to separate SCR larval stages.

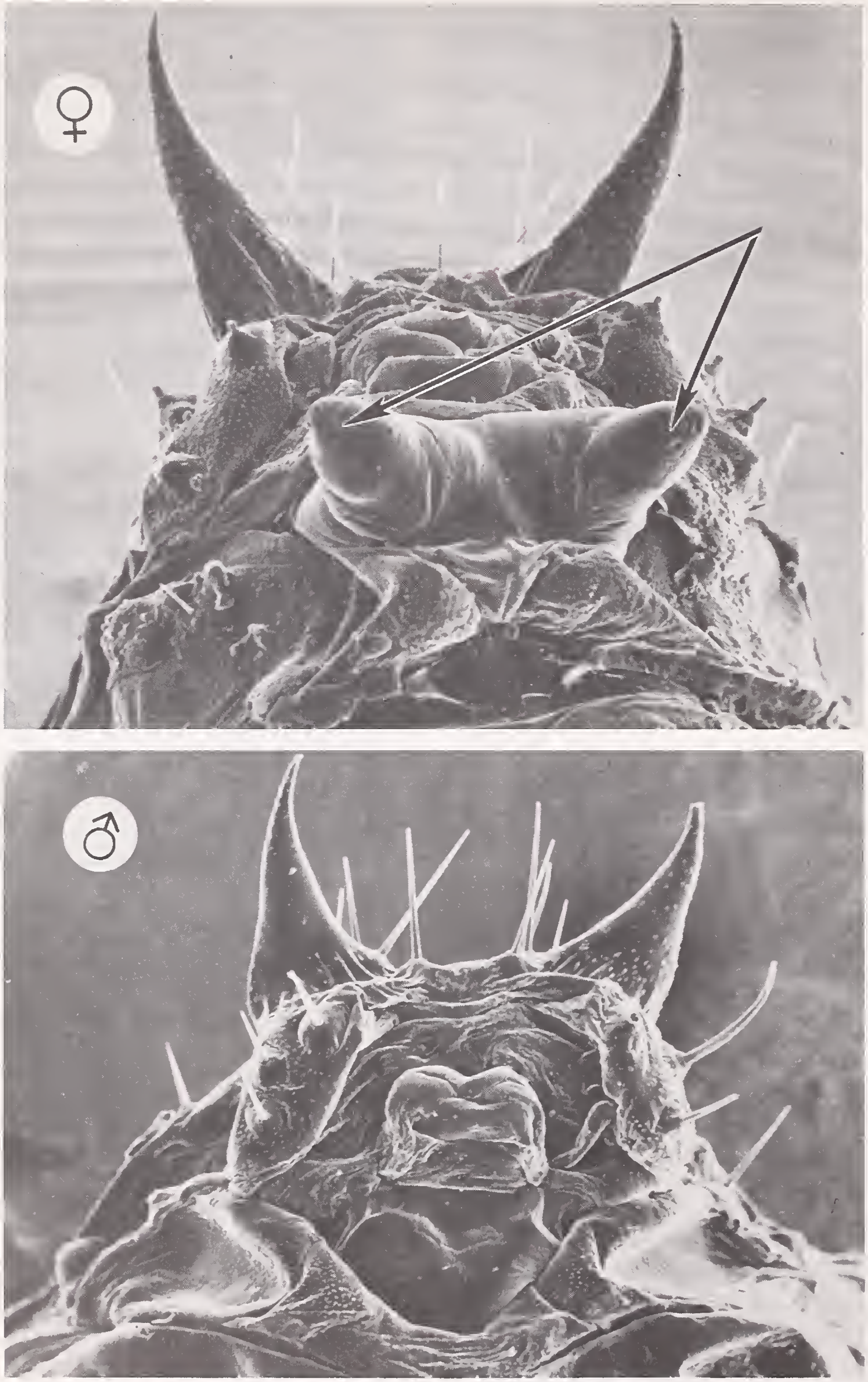

FIGURE 22.—Differences in the ventral abdominal structures of the pupae of female and male corn rootworms. Arrows indicate the papillae found only on females.

DISEASES ASSOCIATED WITH LABORATORY REARING

Unidentified fungi and bacteria have sporadically been observed in laboratory colonies of corn rootworms. However, these organisms are not true pathogens but merely invade the hosts because of such adverse conditions as overwatering of the plant substrate or excessively high relative humidity.

Protozoa

Within the past several years, a disease problem has been encountered which has caused problems in maintaining an efficient corn rootworm rearing program. The disease is caused by a gregarine (Protozoa: Eugregarinida) that appears cyclically in the adult population. These parasites appear within the gut lumen and according to Weiser (*35*), their degree of "pathogenicity" has not been clearly established. Apparently the SCR and WCR are infected in the laboratory colonies by the same gregarine.

Studies indicate that this disease is transmitted in the adult stage when a beetle ingests one or more spores. Other stages of the corn rootworm have been examined and no recognizable stage of the parasite has ever been detected.

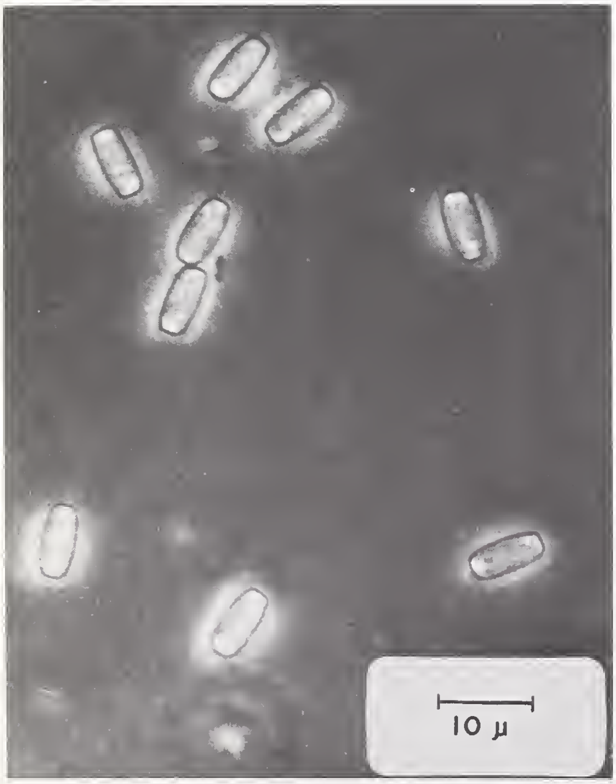

FIGURE 23.—Gregarine spores as observed with phase contrast microscopy.

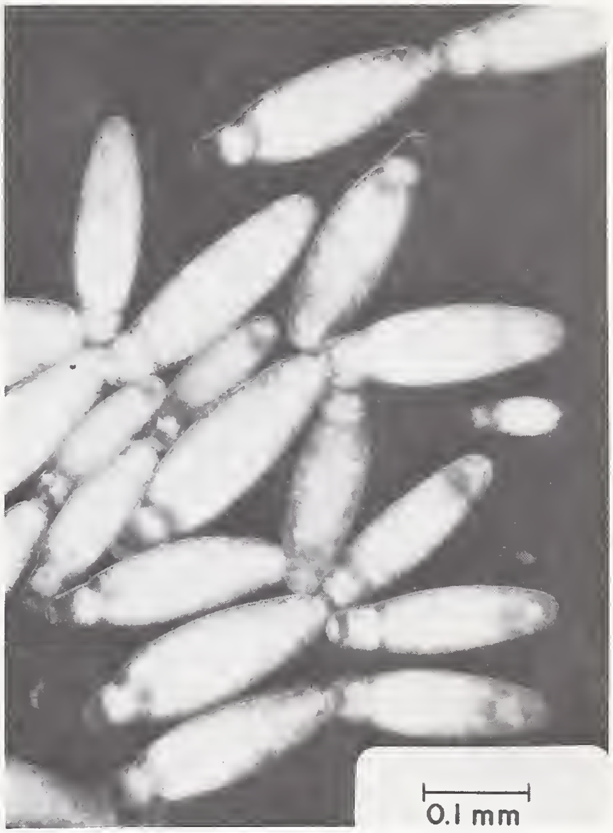

FIGURE 24.—Gregarine sporant stage. Note numerous pairs in syzygy.

The infection begins when beetles ingest one or more spores (fig. 23). These spores measure 4.5 by 8.0 microns and can be airborne. Within each spore are sporozoites (which are nearly submicroscopic and vary in number according to gregarine species) that are liberated in the gut lumen by disruption of the spore coat.

According to Jahn (*24*) and Hall (*20*), the sporozoites invade or partially invade the epithelial cells of the midgut and become trophozoites. The trophozoites greatly increase in size and eventually differentiate into three segments, that is, the epimerite which is used for attachment to the plasma membrane, the protomerite (middle section), and a deutomerite or posterior section. When differentiation has occurred, the parasite is then called a sporant (fig. 24) and is released into the gut lumen. This stage is used for diagnosing infections.

After dissociation with the epithelial cells, the sporants lose the epimerite. Frequently, two sporants can be observed in tandem (syzygy) which is the beginning of the reproductive process. Once in

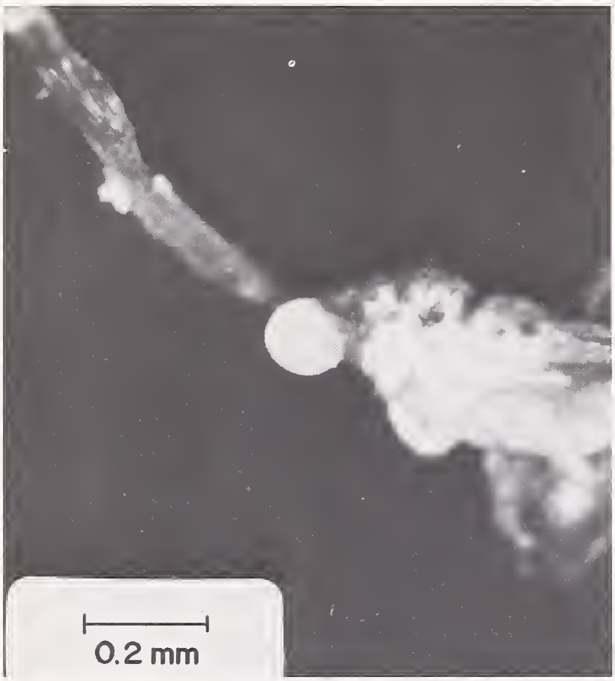

FIGURE 25.—Gametocyst attached to fecal material.

syzygy, the two sporants, at a certain stage of development, become enfolded and secrete a membrane to form a gametocyst (fig. 25). Generally this stage is contained in fecal material and with some effort can be detected in frass as another diagnostic means for detecting this disease. Gametogenesis then occurs within the gametocyst by fission of the microgametes and macrogametes.

In some species of gregarines, mature spores are formed within 3 to 4 days when held at greater than 50 percent RH (*24*). The maturation period has not yet been established for this gregarine; however, it could be an important factor in reducing spore incidence in a rearing area.

Numerous (hundreds) spores are released from each cyst to become airborne and capable of infecting other beetles. Therefore, rearing areas must be kept as clean as possible. At present, the only method for dealing with this parasite is daily cleaning of adult cages, for example, removing all dead beetles and frass within each cage and by vacuuming frass and debris that collect underneath each cage.

Periodic (weekly) scrubbing of the walls of the adult rooms with an antiseptic such as 10 percent bleach is also done; however, the actual value of this effort is unknown. Fumigating the rearing room with methyl bromide reduced the disease for several months; however, the lack of isolation of this room from contaminated areas prevented keeping the colony free of gregarines.

Apparently this parasite, when present in high numbers in a laboratory colony, reduces overall colony vigor, particularly fecundity rates. Whenever cages of newly emerged adults are placed in contaminated areas, they frequently suffer high mortality and low fecundity rates within 2 to 4 weeks or at about the time peak egg production is expected. A definite pathogen/host relationship must be established before determining how much effort, materials, and rearing facility modifications must be made to offset the detrimental effect these parasites have on laboratory colonies.

Any laboratory which intends to establish a colony from field-collected adults should be alert to this disease problem. Surveys over wide areas indicate that up to 100 percent of the field populations of both WCR and NCR can be infected, particularly in the later part of the adult season. Caution should also be exercised whenever field-collected adults are brought near a pre-existing laboratory colony.

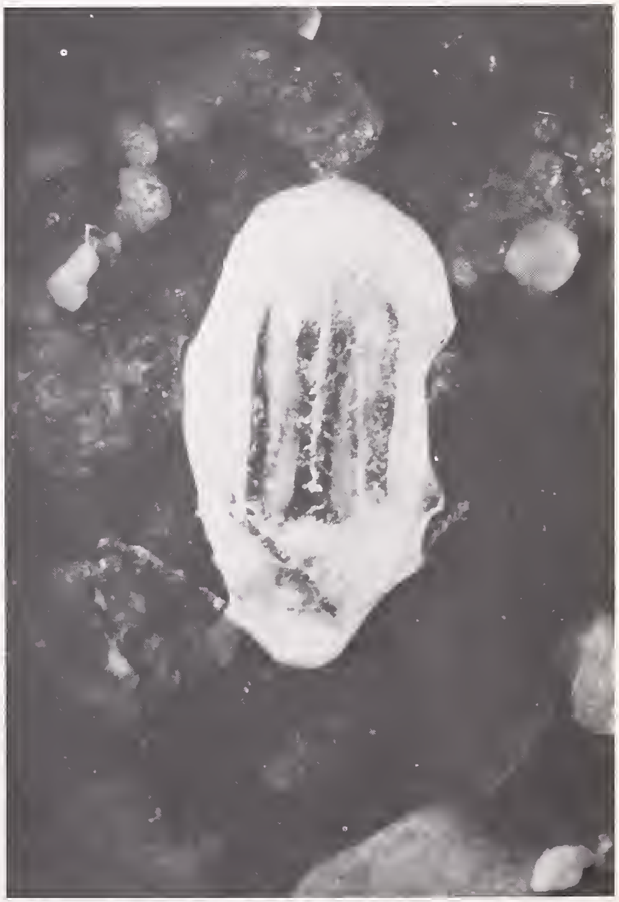

FIGURE 26.—Western corn rootworm beetle killed by *Beauveria bassiana*.

Another protozoan disease in corn rootworm populations has been detected (J. V. Maddox, personal communication).[5] This protozoan, a microsporidian, was more prevalent in the NCR. Geographically the incidence of the disease is higher in populations in Southern Illinois and Missouri and less common in the more northern collections. Surveys of South Dakota populations of WCR and NCR have not indicated the presence of this microsporidian.

Fungi

An additional entomophagous pathogen, the fungus *Beauveria bassiana* has occasionally been observed causing beetle mortality (fig. 26). This pathogen is ubiquitous in nature and can become more of a problem whenever field-collected adults are brought in contact with a laboratory colony or whenever the relative humidity in the rearing area is higher than 75 to 80 percent. Within this rearing scheme, where temperature and relative humidity is maintained within limits, the incidence of this fungus has not been significant. In other regions of the Corn Belt, where humidity is generally higher, this fungus could potentially be a problem.

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[5] J. V. Maddox, unpublished communication. Illinois Natural History Survey, Urbana, IL 61801

SCHEMATIC OUTLINE OF REARING PROCEDURE

Western Corn Rootworm

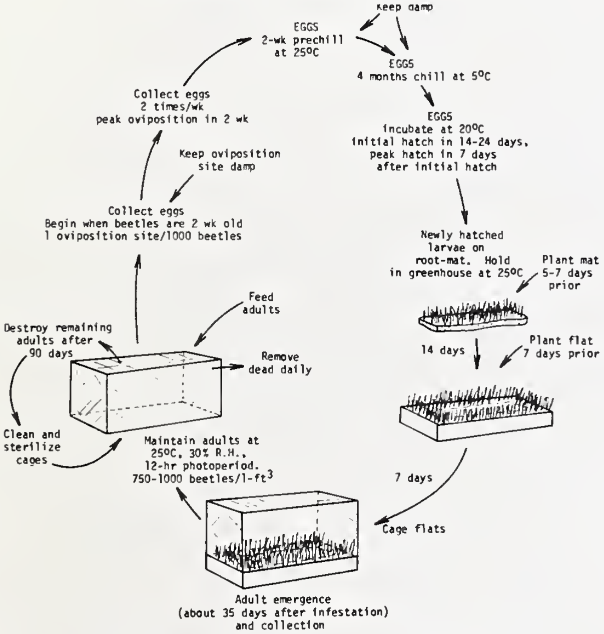

FIGURE 27.—Schematic outline of rearing procedure for western corn rootworm.

Southern Corn Rootworm

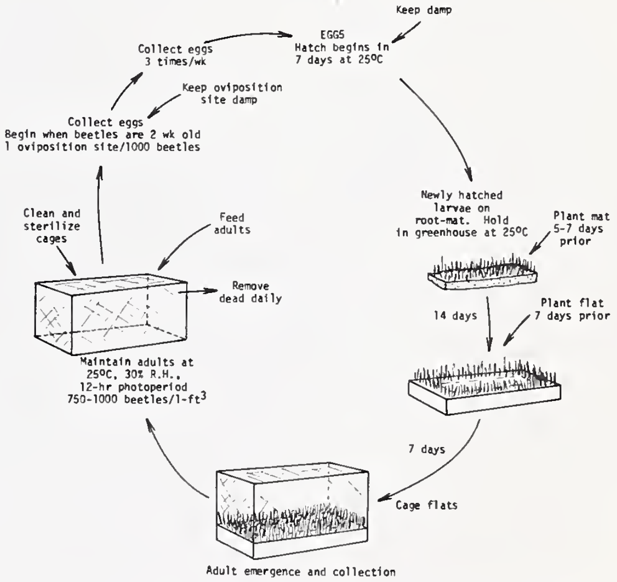

FIGURE 28.—Schematic outline of rearing procedure for southern corn rootworm.

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